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FOOD-INDUCED SIZE-SPECIFIC MOLT SYNCHRONY OF THE SAND CRAB, *EMERITA ANALOGA* (STIMPSON)

PAUL R. SIEGEL

Department of Biological Sciences, University of California, Santa Barbara, California 93106

ABSTRACT

Synchronous molting was found in both laboratory and field populations of the intertidal, filter feeding anomuran *Emerita analoga*. Molt synchrony resulted in distinct peak and trough molting periods which were apparently independent of lunar phase and not pheromonally entrained. The intermolt period for *Emerita* was correlated with animal size.

Laboratory experiments showed that the molt cycles of a previously synchronized group of female *Emerita analoga* could be desynchronized and resynchronized by altering feeding regimes.

It is proposed that in nature the increase in phytoplankton, which characterizes the spring bloom, entrains the synchronous molting rhythm observed in the field.

Synchrony tends to be obscured in field samples composed of broad size ranges because of the size-specific character of the intermolt period. For animals which molt synchronously, estimates of growth rate based on molt frequency data extrapolated from field samples can be variable and misleading.

INTRODUCTION

Generally, molt synchrony is considered to be a consequence of selective pressures which result in either the improved adaptation of an organism to its physical environment or a reduction of molting related mortality (Reaka, 1976). Synchronous molting has been recognized in a number of marine Crustacea, and many studies have been undertaken to determine how the molt cycles of animals in a population become synchronized. In many species, physical factors such as water temperature (Carlisle, 1953; Dall, 1965; Webster, 1981), photoperiod (Aiken and Waddy, 1976; Conan, 1984), lunar phase (Braley, 1979; La Hue, 1981), and tidal cycles (Klapow, 1972; Reaka, 1976) have been implicated as synchronizers ("zeitgebers"). In others, molting cycles have been synchronized by biological factors such as pheromones (Howe, 1981), the interaction of growth and reproductive hormone systems (Scudamore, 1948; Conan, 1984), the periodic availability of food (Joose and Testerinck, 1977), or synchronous hatching (Dagg, 1976).

During studies on the role of food availability on molt synchrony it has been difficult to determine whether synchronous feeding was a cause or a result of molt cycle synchronization (Klapow, 1972). Crustacea generally do not feed during certain phases of the molting cycle (Passano, 1960); therefore, the cyclic presence or absence of food in the gut of an animal under study can be confusing.

In the present study, the molting synchrony of the sand crab, *Emerita analoga*, was investigated and the role of various physical and biological phase setters explored.

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Present address: Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania, Africa.

It is proposed that the observed molt synchrony was a ramification of energy partitioning and not a direct consequence of selective pressures.

Materials and Methods

General collection, measurement, and maintenance techniques

Female *Emerita analoga* were collected from the intertidal zone of Goleta Beach, 15 km west of Santa Barbara, California. Samples containing sand and sand crabs were scooped from the beach by hand or by shovel, placed in 1 mm mesh bags and rinsed in the ocean to separate the crabs from the sand. The carapace length of crabs was determined using the method of Wenner *et al.* (1974), and a narrow size class selected for each experiment. The actual carapace length range of the size class selected for each experiment depended upon the size of crabs available on the beach at the time of collection. Animals were kept in continually flowing unfiltered sea water in circular tanks. The bottoms of the tanks were covered with 5 cm of sand in which animals could burrow. The tanks were checked daily for molts by stirring up the sand, which caused the exuvia to rise to the surface of the sand. Molt frequencies percentages were based on the number of animals alive in a tank at the end of each week.

Long term molt frequency of animals in the laboratory: 1979–1980

Eight hundred female *Emerita analoga* (carapace length 10–13.5 mm) were collected in September 1979 and measured by the above methods. Three hundred of the animals were placed in each of two circular tanks (Tanks 1, 2) and two hundred in a third (Tank 3). On 26 March 1980, 23 male *E. analoga* were added to Tank 3.

On 15 August 1980, the size and molt frequency (since the time of collection) of animals in Tanks 1 and 2 were compared. A Student's *t*-test indicated that the size of the animals in the two tanks was not different ($P > 0.1$), and a Mann-Whitney U test indicated that the molt frequency of the animals was likewise not different ($P > 0.1$). The animals in the two tanks (Tanks 1 and 2) were combined, and 106 of the crabs were removed for another experiment, leaving 52 animals which continued to be monitored in this experiment. This group of females was kept apart from males to preclude egg production.

Egg production by the females in Tank 3 (the tank to which males had been added) began in the spring of 1980. Ovigerous females were identified by the presence of a large external egg mass on the pleopods. The percentage of females with egg masses was checked every two weeks.

Intermolt period for laboratory animals: 1981

In January 1981, 150 female *Emerita analoga* were collected from Goleta Beach, measured as previously described, and divided into six size classes, three size classes of large animals (with 30 animals each) and three size classes of small animals (with 20 animals each) (Table I). Two size classes, a small and a large, were placed in each of three tanks. The animals in the small size classes were marked with a small hole in the lower left margin of the carapace, and the animals in the large size classes were marked with a hole on the lower right margin of the carapace. In this way the size class from which a molted animal came could be easily recognized. Molts were collected daily and molted animals were remarked. Molting of the animals in each size class in each tank was monitored through two molting cycles. Each cycle was tested for normality with a Lilliefors' test (Conover, 1980) and the mid-point of each cycle determined graphically (Harding, 1949). The intermolt period of each size class

TABLE 1

Mean carapace length, number of animals per size class, mean time at first and second molting peaks, the percentage of animals molting during each peak, and the intermolt period for the size class as determined by the time between peaks

Location	Carapace length (mm + S.D.)	Mid-point 1st peak	n	% Molt/ peak	Mid-point 2nd peak	n	% Molt/ peak	Intermolt period
Tank 1								
Large	12.2 + 0.8	5 May	21	86	17 June	17	94	43
Small	10.6 + 0.4	7 May	13	115	13 June	9	100	37
Tank 2								
Large	13.5 + 0.4	7 May	27	89	20–21 June	24	104	44.5
Small	11.0 + 0.3	5 May	15	100	13 June	14	86	39
Tank 3								
Large	12.4 + 0.8	4 May	26	62	14 June	27	100	41
Small	10.4 + 0.5	10–11 May	17	89	15 June	15	100	36.5

was determined as the time between the mid-points of the molting peaks (as in Fig. 1). The percentage of animals to molt during a peak was determined by dividing the number of molts by the number of animals alive at the end of the peak.

Field molt frequency and estimated intermolt period: 1982

Estimates of molt frequency and intermolt period of female *Emerita analoga* from the field were calculated to determine how consistent such estimates would be for a synchronously molting species. The estimates were derived using the instantaneous growth rate rationale of Fusaro (1977). From July to September 1982, I collected 13 field samples from Goleta Beach. Each sample consisted of between 51 and 209 animals with 10–12.5 mm carapace length. In all, 1623 animals were collected. After

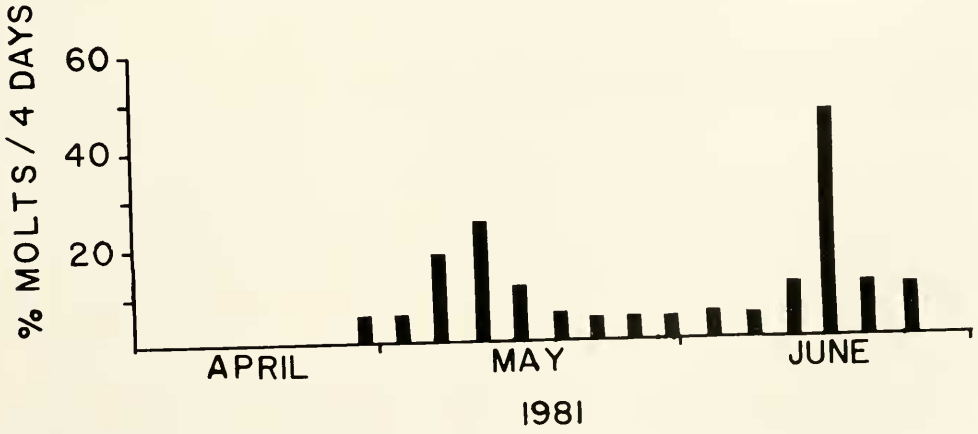

FIGURE 1. Determination of the intermolt period of a single size class of *Emerita analoga* held in the laboratory. The example shown is based on the molt frequency of the small size class from Tank 1 (Fig. 4) from April to June 1981. Molt frequency was monitored through two molt cycles. Each cycle was tested for normality and the intermolt period defined as the time between the mid-points of the two peaks.

capture, animals were kept in the laboratory in trays, maintained in flowing sea water for four days, and checked daily for molts. The intermolt period estimates (Table II) were derived from the formula $I = t/Pt$ where I was the intermolt period, t was the number of days that animals were in the laboratory (4), and Pt was the proportion of animals from the sample to molt during time t (Fusaro, 1977). For example, if 20% of the animals molted in 4 days, the estimated intermolt period would be 4/0.2 or 20 days.

Food as a synchronizer of molting: 1982–1983

On 27 September 1982, 90 female *Emerita analoga* (carapace length = 11.6 ± 0.7 mm) were collected, measured, and divided equally between three tanks (A, B, C). The tanks were supplied with a constant flow of filtered (plankton-free) sea water, thereby restricting the crabs' feeding. The animals were starved in that manner for a period of six weeks. On 9 November 1982, I began feeding the animals in Tank A each day. Two weeks later (24 Nov 1982) I began daily feeding of the animals in Tank B. Food consisted of 3.4 g of *Artemia salina* eggs hatched in 9.5 litres of sea water at 28°C for 48 hours. The animals in Tank C remained unfed for the entire experiment as a control. Tanks were checked daily for exuvia.

Feeding of the animals in both Tanks A and B was discontinued on 24 January 1983 and the animals starved for six weeks. The daily feeding of both tanks was resumed on 10 March 1983 and continued until the end of the experiment on 14 May 1983. The temporal distribution of molting events in the tanks was compared using a Smirnov test (Conover, 1980).

Results

The molt frequency of female *Emerita analoga* from January 1979 to December 1980 was characterized by significant ($P < 0.01$) peaks and troughs (Fig. 2A). Molt frequency was low during the winter and fall, resulting in broad, ill defined cycles, whereas cycles during the spring and summer were quite distinct. There were three molting cycles between April and September 1980, with approximately 100% of the animals molting during each cycle. Up to 32% of the animals molted per week during

Table II

Estimated intermolt periods based on the molt frequency of animals collected from the field and held in the laboratory for 4 days

Collection	n	% Molt/4 days	Estimated intermolt (days)
27 June	59	3.4	117.6
10 July	185	1.1	363.6
15 July	165	14.5	27.6
21 July	147	6.1	65.6
28 July	114	5.3	75.5
1 Aug	51	0.0	
7 Aug	77	9.1	44.0
13 Aug	87	8.0	50.0
18 Aug	102	1.0	400.0
25 Aug	161	14.9	26.8
31 Aug	209	4.8	83.3
8 Sept	131	2.3	173.9
22 Sept	135	0.7	571.4

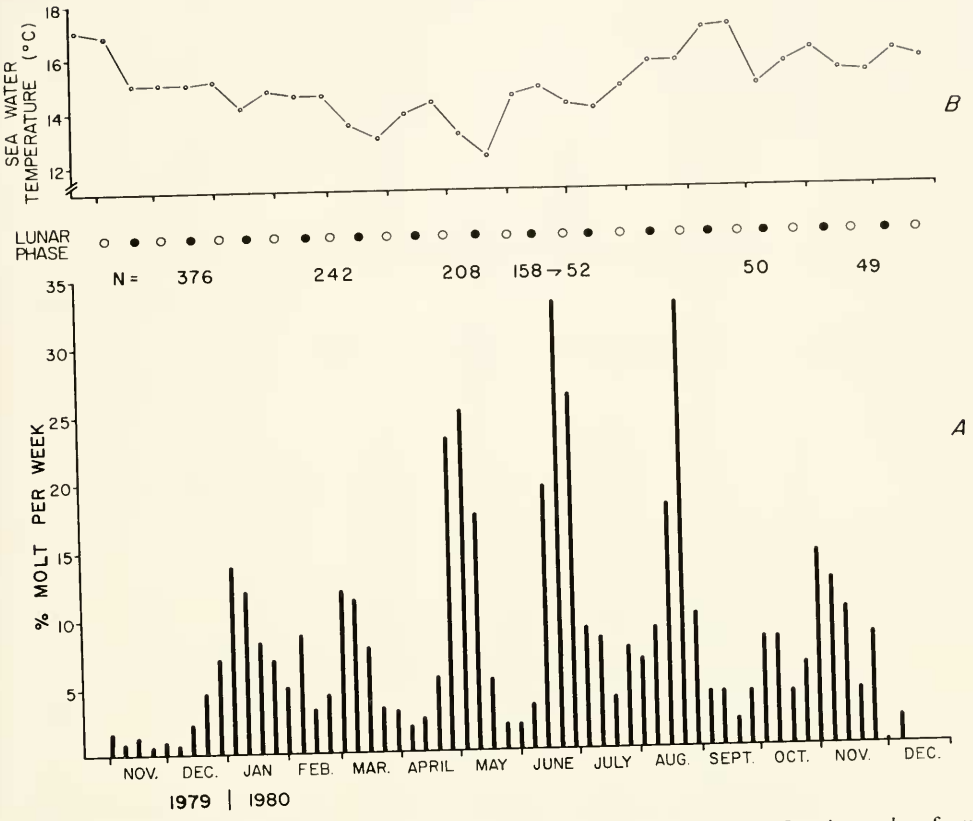

FIGURE 2. (A) Molt frequency (% molts/week) of a tank of female sand crabs, *Emerita analoga* from November 1979 to December 1980. Superscripted numbers represent the number of crabs upon which the percentages were based. The reduction in sample size in June-July was due to removal of some crabs for another experiment (see text for explanation). (B) Also shown are lunar phases (open circles = full moon, closed circles = new moon) and mean sea water temperature for the same period.

peak periods compared to 2% molting per week during trough periods. An analysis of molt frequency comparing molt frequencies during full and new moon lunar phases (Fig. 2B) to the molt frequency during first and third quarter lunar phases showed no significant difference ($P > 0.1$).

Molting cycles during the spring and summer of 1980, for females which produced eggs and equal-sized females which did not produce eggs, were characterized by three peaks: one in early May, one in late June, and one in mid-August (Fig. 3). During the same period, the percentage of ovigerous females in the tank to which males had been added increased to 95.5%, with no disruption of the synchrony phasing between the two groups.

The cyclic nature of the molt frequency was used to determine the intermolt period for six different size classes of female *Emerita analoga* (Table I, Fig. 1). A Kendall's tau test revealed that the intermolt period was positively correlated with carapace length ($P < 0.01$) (Fig. 4).

The results from the 1982 field samples, which were undertaken to detect molt synchrony in a field population, are shown in Figure 5. The percentage of females

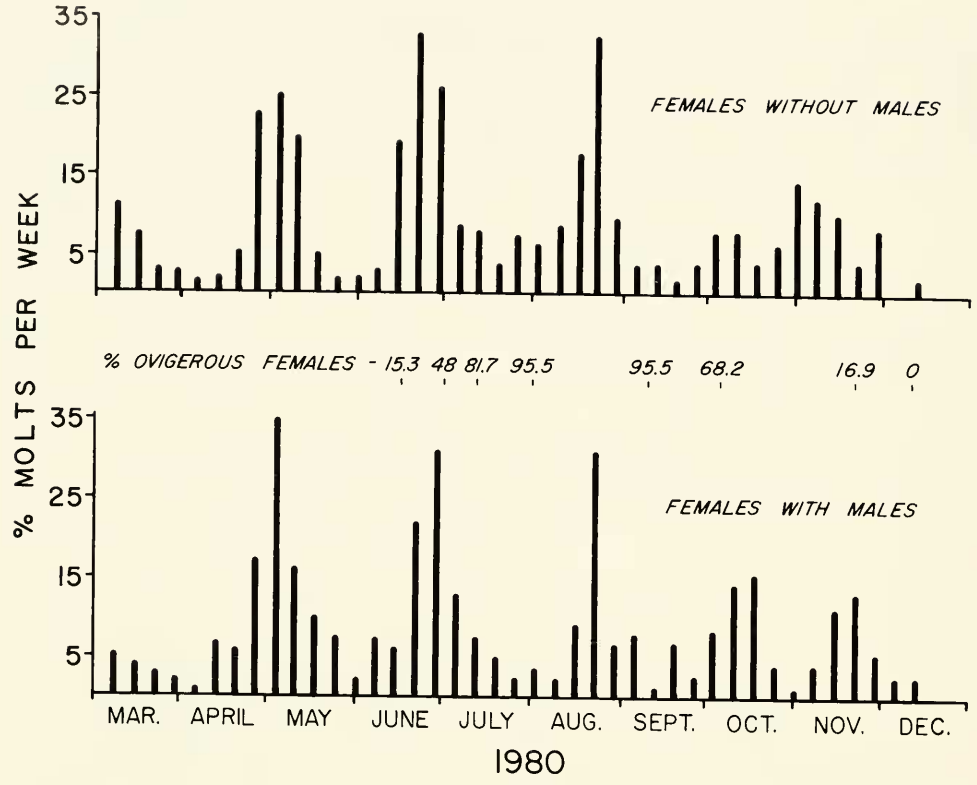

FIGURE 3. Molt frequency (% molts/week) of female sand crabs during the reproductive season of 1980. Some females were kept without males and so did not produce eggs (upper histogram) while some females kept with males (lower histogram) did produce eggs. The percentage of females to carry extruded eggs is shown between the two histograms for each date that egg production was assessed.

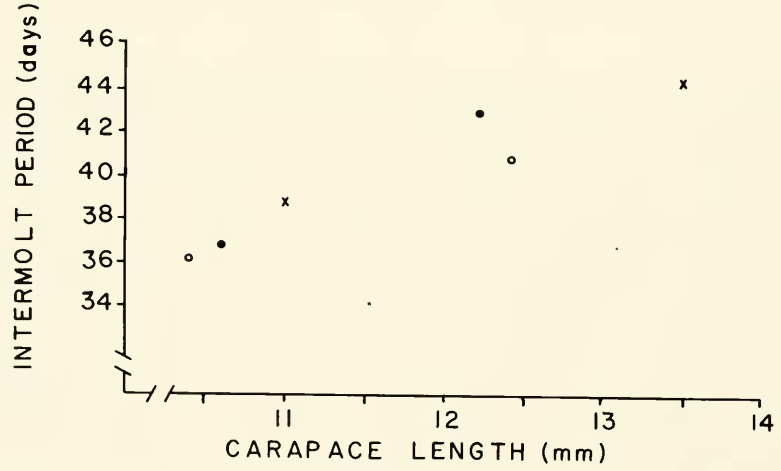

FIGURE 4. Intermolt period as a function of carapace length. Intermolt periods shown for three tanks of female *Emerita analoga* (O, ●, ×), with two size classes in each.

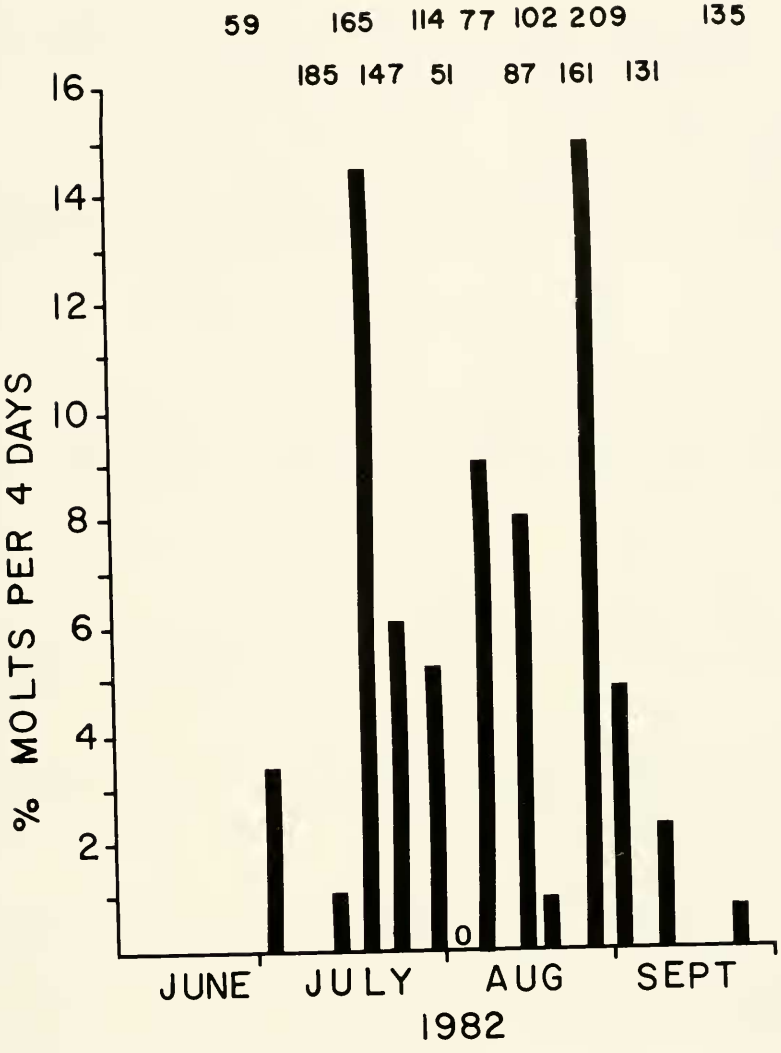

FIGURE 5. Molt frequency of female sand crabs *Emerita analoga* collected from Goleta Beach between July and September 1982. A total of 1623 animals was collected. Superscripted numbers represent the sample size upon which the % molts/4 days were based.

which molted in the laboratory over each four day holding period showed peaks and troughs which varied significantly from random ($P < 0.001$). Nearly 15% of animals collected on 15 July and 25 August molted during the four day period that each sample was in the laboratory. In contrast, less than 2% of the animals collected on 10 July, 1 August, and 18 August molted during each respective four day period. Estimates of the intermolt period (the number of days necessary for 100% of the animals to molt) for female *E. analoga* from the field ranged from less than a month to more than a year (Table II), even though the animals from each sample were the same size.

The molt cycles of animals collected from the field could be desynchronized and resynchronized using food as a desynchronizing/resynchronizing agent (Fig. 6). After

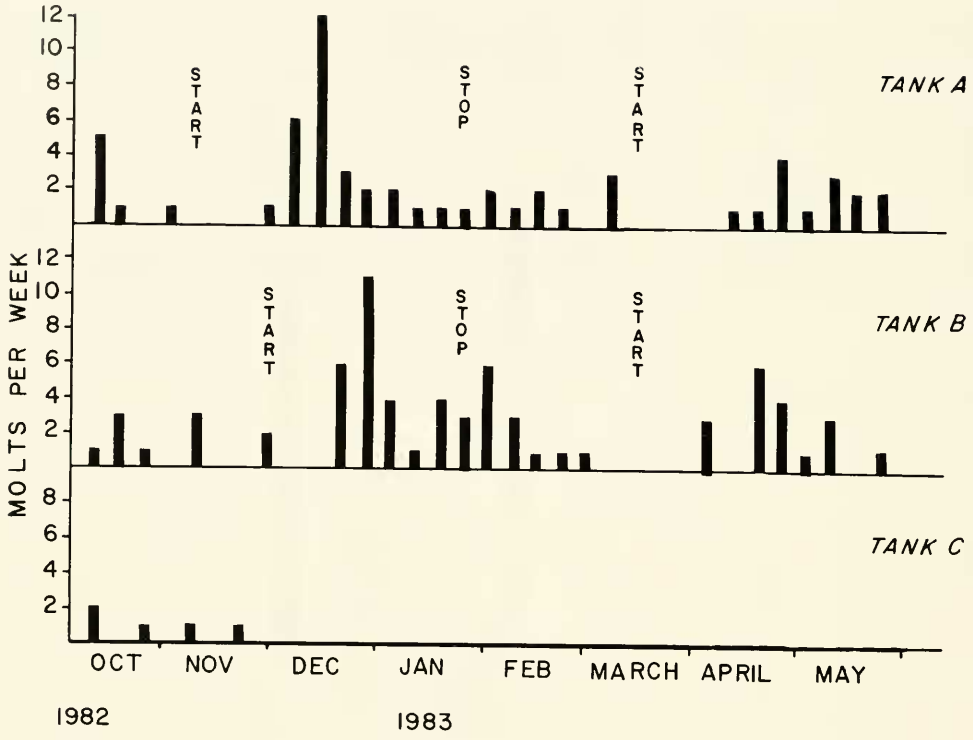

FIGURE 6. Desynchronization and resynchronization of the molt cycles of female *Emerita analoga* in response to different feeding schedules. Crabs were starved until feeding was begun (START). Daily feeding was continued until STOP and animals remained unfed until the following START. Animals in Tank C were unfed for the duration of the experiment.

the initial period of starvation and the different feeding schedules which followed, molting peaks occurred in the two tanks (Tanks A and B) two weeks apart, corresponding to the initial separation of feeding ($P < 0.05$). However, after the animals in both tanks were again starved for six weeks and feeding begun for the animals in both tanks at the same time, there was no longer a statistically significant difference in the timing of molting ($P > 0.1$).

DISCUSSION

Models of crustacean growth and production contain terms for size-specific growth rates which are derived from molt frequency and molt increment estimates. The molt frequency of field populations are often derived from the proportion of soft shelled animals caught during field samples (Conan *et al.*, 1976; Efford, 1967) or from short term laboratory experiments (Fusaro, 1978). However, unless the distribution of molting events through time is random (or constant) estimates of the molt frequency based upon such measures may greatly over or under estimate the real molt frequency. As a result of synchronous molting of female *Emerita analoga* (Fig. 5), estimates of the intermolt period varied by more than an order of magnitude (Table II).

From the preceding experiments I concluded that neither the physical factors of temperature, nor lunar phase, nor the biological factors of reproductive seasonality or pheromones played a pivotal role in the establishment or maintenance of the molt

synchrony of *Emerita analoga*. The degree to which animals were synchronized (peak amplitude) increased between April and May 1980, while the ambient sea water temperature was decreasing, and remained high while temperature both decreased and increased (Fig. 2). Periods of low molting were not associated with periods of low temperature nor did periods of high molting occur when the sea water temperature was highest. Clearly, the molting of *E. analoga* was not synchronized by the rise of sea water temperature above a physiological threshold, as has been shown for *Leander* (Carlisle, 1953), *Metapenaeus sp.* (Dall, 1965), or *Palaemon elegans* (Webster, 1981). The duration of each molting cycle was too long to be a response to semi-lunar periodicity, and statistical analysis revealed no lunar periodicity. The intermolt period for the female *Emerita analoga* represented in Figure 2 appears to reflect a bimonthly period but this was an artifact of the size class (12 mm carapace length) of the crabs (Fig. 4).

Molt synchrony was also not entrained as a consequence of egg production (Fig. 3). Females in both egg producing and non egg producing tanks showed highly synchronized molting months before the onset of egg production (June 1980). The fact that molt synchrony was uneffected by egg production was also evident from the simultaneous molting which occurred in both of those tanks ($P > 0.1$ in all cases). Synchrony was maintained between those two groups even as egg production increased, because the development time for eggs was less than the intermolt period of the females (time between peaks). Females which produced eggs molted at the same time as females which did not and produced a brood of eggs which matured and hatched before the subsequent molting peak. In this way, molting between the tanks remained synchronized without egg loss.

Molt synchrony was apparently not mediated by the action of a pheromone, as has been proposed for the synchronization of molting of *Macrobrachium rosenbergii* (Howe, 1981). In general, pheromonal induction of molt synchrony occurs when a molting animal sheds a pheromone into surrounding water, which triggers a molting response from conspecifics in the vicinity. If this has been the mechanism whereby the molt cycles of *Emerita analoga* were synchronized, then the two size classes of female crabs that were in the same tank (Fig. 4) would have tended to molt at the same time. Pheromonally induced synchrony would have resulted in simultaneous molting peaks for different size classes of animals in the same tank, and an intermolt period for the two size classes which would have been identical. Instead, the intermolt period for the six size classes shown in Figure 4 varied as a function of carapace length (Table I). A positive correlation between carapace length and intermolt period is common among decapods (Hartnoll, 1982; Mauchline, 1977) so it was not surprising to find that *E. analoga* followed that pattern.

The data presented in Figure 6 showed that the molt cycles of animals of a fixed size class could be desynchronized and resynchronized. Both desynchronization and resynchronization were induced by altered feeding schedules following a period of starvation. My interpretation of those results is that molt synchrony was a ramification of energy partitioning. The reasoning is as follows.

The energy assimilated by a non-reproductive animal is, for the most part, partitioned between growth and metabolism, and metabolic energy demands frequently vary with animal size (Vidal, 1980; Ross, 1982). If the molt frequency of the animals were determined by ration, which is common among crustacea (Hartnoll, 1984), the energetic requirement for molting would be met by all the like-sized individuals of a starved group at about the same time after food became available resulting in a pulse of molting.

Since the molt frequency of *Emerita analoga* is strongly affected by ration (Fusaro, 1977) and since sand crabs feed predominantly on phytoplankton (Osorio *et al.*, 1967), the synchronous molting pattern shown in Figure 2 can be interpreted through the theoretical framework described above. Although no phytoplankton abundance data were collected during the spring of 1979 to directly correlate the onset of the spring bloom with the onset of the spring through summer molting peaks, phytoplankton abundance off the coast of Central California is generally high during spring and summer and low during fall and winter (Eickstaedt, 1969), although occasional plankton blooms may occur at the end of the year, resulting in greater than normal sand crab growth (Siegel and Wenner, 1984). The period of highest phytoplankton abundance corresponds to the period of maximum growth of *E. analoga* while sand crab growth during the fall and winter (when phytoplankton levels are normally low) is characteristically slow (Efford, 1967; Fusaro, 1977).

During the winter (when food is normally sparse) sand crabs apparently use reserves gathered during the preceding season, and both molt frequency and degree of synchrony are low. The spring bloom, which characteristically occurs in March or April (Eickstaedt, 1969), may have provided the animals, which had been starved during the winter, with a pulse of food which was reflected in an increase in both molt frequency and degree of synchrony. The synchronous molt cycles were maintained throughout the summer since the females were the same size and had the same intermolt period, but, as food levels dwindled towards winter, both molt frequency and the degree of synchrony exhibited by the population decreased.

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